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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: Shih-Chieh Hung et al. Art Unit: 1636
Application No.: 09/761,893 Examiner: Jennifer Dunston
Filing Date: 01/17/2001
Title: Method of isolating mesenchymal stem cells

APPEAL BRIEF PURSUANT TO 37 C.F.R.§41.37

Commissioner for Patents
Mail Stop Appeal brief –Patents
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Sir:

As set forth in the Notice of Appeal filed on March 12, 2012, appellants hereby appeal the examiner's rejection of claims 1, 4, 6, 9-11, 34-35, 38 and 43-45 of the above-identified application.

Appellants respectfully request that the Board of Patent Appeals and Interferences reverse the rejection of these claims.

(i) *Real party in interest.*

The real party in interest is the appellants, Shih-chieh Hung and Wai-hee Lo,

(ii) *Related appeals and interferences.*

There are no related appeals or interferences pertaining to the above-identified application.

(iii) *Status of claims.*

Claims 2-3, 5, 7-8, 12-33, 36-37, 39-42 have been canceled or withdrawn.

Claims 1, 4, 6, 9, 11, 34, 35 and 38 have been rejected in the Office Action mailed on November 17, 2011 under 35 U.S.C. §103(a) as being unpatentable over U.S. Patent 5811094 to Caplan et al. (Caplan) in view of U.S. Patent 7374937 to Prockop et al (Prockop) and U.S. Patent 4871674 to Matsui et al (Matsui). The claims have also been rejected in the same Office Action under 35 U.S.C. §103(a) as being unpatentable over Caplan in view of Wheater's Functional Histoolgy (1993) to Burkitt et al (Burkitt) and Matsui. The same Office Action further rejected the claims under 35 U.S.C. §103(a) as being unpatentable over Caplan in view of U.S. Patent 5077012 to Guirguis (Guirguis) and Matsui.

Claims 1, 4, 6, 9, 11, 34, 35 and 38 were previously rejected in the Office Action mailed on April 6, 2011 as being unpatentable over Caplan in view of U.S. Patent Prockop and Matsui, as well as Caplan in view of Burkitt et al and Matsui; Caplan in view of Guirguis and Matsui.

Calims 43-45 were withdrawn from consideration in the Office Actions mailed on November 17, 2011 and January 11, 2012.

As claims 1, 4, 6, 9, 11, 34, 35 and 38 have clearly been twice rejected, an appeal of all pending claims is appropriate under 35 U.S.C. § 134(a). The rejections/objection of claims 1, 4, 6, 9-11, 34-35, 38 and 43-45 are being appealed.

(iv) *Status of amendments.*

Claim 1 was amended after Final Rejection mailed on November 17, 2011. However, the Office Action mailed on January 11, 2012 also rejected the claim. The appellants believe that the amendment presenting rejected claims in better form for consideration on appeal under 37C.F.R. §1.116.

(v) *Summary of claimed subject matter.*

In fulfillment of the provisions of 37 C.F.R. §41.37(c)(1)(v), appellants provide a summary of the claimed subject matter for each of the independent claims at issue. The present application currently presents one pending independent claim (claim 1).

Claim 1 is directed to a method for isolating mesenchymal stem cells from bone marrow aspirate (paragraphs. [0002], [0009] and [0010]), comprising:

(a) providing a cell mixture comprising mesenchymal stem cells and other cells; (paragraphs [0009], [0023] and [0024])

(b) seeding and culturing (paragraphs [0009] and [0023]) the cell mixture in a culture device (paragraphs [0009], [0023], [0024] and [0025]) comprising an upper plate with pores and a lower plate base, said upper plate made of a mesenchymal stem cell adhering material with pores of about 0.4 to 40 microns in diameter, where mesenchymal stem cells adhere and are cultured, and the lower plate base, where small-sized cells adhere following passing through the pores in the upper plate, said culturing with medium containing factors that stimulate mesenchymal stem cells growth without differentiation and allow for the selective adherence of only the mesenchymal stem cells to the upper plate surface; (paragraphs [0025], [0029] and [0039])

(c) purifying mesenchymal stem cells by removal of haematopoietic stem cells and non-adherent cells on the upper plate by changing medium; (paragraphs [0030], [0034], [0037] and [0039]) and

(d) collecting mesenchymal stem cells from the upper plate by recovering with trypsin-EDTA. (paragraphs [0023], [0029], [0030], [0031] and [0035])

(vi) *Grounds of rejection to be reviewed on appeal.*

Appellants respectfully request that the Board of reverse the rejection of claims 1, 4, 6, 9, 11, 34, 35 and 38 under 35 U.S.C. §103(a) as being unpatentable over Caplan in view of Prockop and Matsui; Caplan in view of Burkitt and Matsui and Caplan in view of Guirguis and Matsui.

Appellants also respectfully request that the Board of reverse the withdrawing from consideration of claims 43-45.

(vii) *Argument.*

35 U.S.C.§103(a) Obviousness Rejections

M.P.E.P. 706.02(j) sets forth the standard for a Section 103(a) rejection:

To establish a prima facie case of obviousness the prior art reference or references combined must teach or suggest all the claim limitations. Additionally, there must be a reason that would have prompted a person of ordinary skill in the relevant field to combine the prior art elements in the manner claimed. Finally, to establish a prima facie case of obviousness, there must be a reasonable expectation of success. Furthermore, the reason that would have prompted the combination and the reasonable expectation of success must be found in the prior art, common knowledge or nature of the problem itself and not based on the Applicant's disclosure. Underlying the obvious determination is the fact that statutorily prohibited hindsight cannot be used.

A. .Obviousness rejection based Caplan in view of Prockop and Matsui

The examiner stated that Caplan taught the isolation of human mesenchymal stem cells from aspirated marrow. According to the examiner, Caplan failed to teach that the mixed population of cells in medium is seeded into a culture device comprising an upper plate and a lower plate base.

To cure the deficiencies of Caplan, the examiner combined Caplan with Prockop and Matsui. Prockop taught RS cells can be separated from non-RS mesenchymal stem cells by ultrafiltration, where the ultrafiltration membrane has 10 micrometer pores. Matsui taught culturing cells in a cell culture device comprising an insert with a membrane filter on the bottom.

The examiner then concluded that it would have been obvious to one of ordinary skill in the art at the time the invention was made to modify the method of isolating mesenchymal stem cells of Caplan to include the introduction of the mixed composition of cells comprising mesenchymal stem cells and medium into culture dish taught by Matsui. One of ordinary skill in the art would further modify Matsui's filter according to the Prockop's teaching.

The examiner, however, has failed to explain why a person of ordinary skill in the relevant field would combine the prior art elements in the manner claimed. Moreover, there was also no reasonable expectation of success. Prockop's disclosure (US 7374937), which was very close to the application date of this application, did mention that "this review of the literature demonstrates that transplantation of MSCs have significant therapeutic and gene transfer uses. However, **prior art methods for isolating MSCs and inducing their proliferation have practical limitations, including the extent of population expansion that can be achieved using prior art methods. There remains a critical need for methods of reliably inducing significant proliferation of**

MSCs in culture without inducing differentiation of the MSCs as they proliferate." Furthermore, Prockop also mentioned that "Despite the great interest in examining the biology of MSCs and their potential use for therapy, **there is still no generally accepted protocol for isolating and expanding MSCs in culture**. Most experiments relating to differentiation of MSCs have been performed using cultures of MSCs that have been isolated primarily by virtue of the MSCs tight adherence to tissue culture dishes, as described (Friedenstein et al., 1976, Exp. Hematol. 4:267-274; Friedenstein et al., 1987, Cell Tissue Kinet. 20:263-272). Others have attempted to prepare more homogenous MSC populations (e.g. Long et al., 1995, J. Clin. Invest. 95:881-887; Simmons et al., 1991, Blood 78, 55-62; Waller et al., 1995, Blood 85:2422-2435; Rickard et al., 1996, J. Bone Miner. Res. 11:312-324; Joyner et al., 1997, Bone 21:1-6). However, none of these protocols has gained wide acceptance. In addition, these protocols have been primarily designed to isolate osteoblast precursors. Use of these protocols has not been investigated to determine if they yield cells that are truly multipotential."

Moreover, Kato et al (US Patent Application 20050013804, filing date: 09/12/2001) also mentioned that "**the conventional culture methods however cannot produce sufficient amounts of mesenchymal stem cells because the proliferation of said stem cells stops or becomes extremely slow around 15th generation.**" Lange et al (US Patent Application 20070160583, filing date: 08/6/2004) also disclosed "from the results known to prior art, it is clear that sprouting of less mitotic cells cannot be prevented with any separation method. Rather the cultivation conditions play a large role here. What is needed here is careful selection of the population with most evident proliferation features." Furthermore, Lin et al (US Patent Application 20070128722, filing date: 12/5/2005) "due to the reason that adult stem cells are rare in adult tissues and it is difficult to expand their numbers in cell culture, methods of proliferating adult stem cells in culture are sought,"

In other words, these above evidence clearly demonstrated that no reason that would have prompted the combination and the no reasonable expectation of success was found in the prior art, common knowledge or nature of the problem itself.

Furthermore, Matsui's disclosue (US patent 4871674) was critized to be "In the use of these cell culture inserts, gases may not be exchanged sufficiently because the area between the sidewall of the insert and the culture plate is too small." (Column 1, line 37 to 39 US patent 5652142). One of the ordinary skill in the art would also run into difficulty to adopt Matsui's device as the upper plate of this application.

The Matsui's 4871674 patent was issued on 10/3/1989. Caplan's 5811094 patent was applied on 4/11/1995 and issued on 9/22/1998. Prockop's 7374937 patent was applied on 10/25/2000 (effective date 3/14/2000). This application was filed on 1/17/2001 with the priority date on 10/17/2000. The time frame also highly did not support the Office Action that "it would have been obvious to one of ordinary skill in the art at the time the invention was made to combine these three prior arts and do some modifications." If it had been obvious, Prockop would have combined the teachings of Matsui and Caplan to reach the method recited in this application. Moreover, Prof. Prockop has been a well known mesenchymal stem cell researcher in the US and the world, but did not reach the method of this application. Thus, it would not be obvious to one of ordinary skill in the art to do the modification as this application claimed.

B. Obviousness rejection based Caplan in view of Burkitt and Matsui

The examiner stated that Caplan taught the isolation of human mesenchymal stem cells from aspirated marrow. According to the examiner, Caplan failed to teach that the mixed population of cells in medium is seeded into a culture device comprising an upper plate and a lower plate base.

To cure the deficiencies of Caplan, the examiner combined Caplan with Burkitt and Matsui. Burkitt taught red blood cells are 6.7-7.7 μ m in diameter and nucleated cells have a diameter greater than 7.7 μ m. Matsui taught culturing cells in a cell culture device comprising an insert with a membrane filter on the bottom.

The examiner then concluded that it would have been obvious to one of ordinary skill in the art at the time the invention was made to modify the method of isolating mesenchymal stem cells of Caplan to include the introduction of the mixed composition of cells comprising mesenchymal stem cells and medium into culture dish taught by Matsui. One of ordinary skill in the art would further modify Matsui's filter according to the Burkitt's teaching.

As discussed above, there was no reason that a person of ordinary skill in the relevant field would combine the prior art elements in the manner claimed and no reasonable expectation of success. Evidences further opposed that a person of ordinary skill in the relevant field would not do the modification. Moreover, due to the criticism by a prior art, a person of ordinary skill in the relevant field would have a difficulty to use Matsui's culture device.

Caplan clearly disclosed that "as a whole, bone marrow is a complex tissue comprised of hematopoietic stem cells, red and white blood cells and their precursors, mesenchymal stem cells, stromal cells and their precursors, and a group of cells including fibroblasts, reticulocytes, adipocytes, and endothelial cells

which form a connective tissue network called "stroma". (Column 7, line 12-16, US Patent 5811094) The red blood cell taught by Burkitt is only one of the components in bone marrow. After the removal of red blood cell from aspirated marrow, a pure population of mesenchymal stem cells has not been isolated. Thus, a person of ordinary skill in the relevant field would not modify Caplan's method in view of Burkitt's teaching.

C. Obviousness rejection based Caplan in view of Guirguis and Matsui

The examiner stated that Caplan taught the isolation of human mesenchymal stem cells from aspirated marrow. According to the examiner, Caplan failed to teach that the mixed population of cells in medium is seeded into a culture device comprising an upper plate and a lower plate base.

To cure the deficiencies of Caplan, the examiner combined Caplan with Burkitt and Matsui. Burkitt taught the removal of red blood cells from a body fluid using a membrane, preferred pore size of 2 micros or less, with a smooth flat surface which is ideal for collection atypical cells from all types of body fluids. Matsui taught culturing cells in a cell culture device comprising an insert with a membrane filter on the bottom.

The examiner then concluded that it would have been obvious to one of ordinary skill in the art at the time the invention was made to modify the method of isolating mesenchymal stem cells of Caplan to include the introduction of the mixed composition of cells comprising mesenchymal stem cells and medium into culture dish taught by Matsui. One of ordinary skill in the art would further modify Matsui's filter according to the Burkitt's teaching.

Again, as discussed above, there was no reason that a person of ordinary skill in the relevant field would combine the prior art elements in the manner claimed and no reasonable expectation of success. Evidences further opposed that a person of ordinary skill in the relevant field would not do the modification. Moreover, due to the criticism by a prior art, a person of ordinary skill in the relevant field would have a difficulty to utilize Matsui's culture device.

Guirguis disclosed "an apparatus for collecting biological fluids and holding samples taken from a biological fluid for qualitative and quantitative testing." (Abstract, US patent 5077012) Guirguis's apparatus was "for detecting disease markers both for screening as well as for a reference laboratory setting." (Column, 1 line 15 to line 17) Thus, a person of ordinary skill in the relevant field would not modify Caplan's method in view of Guirguis's teaching.

Unexpected result

Prockop also mentioned that "despite the great interest in examining the biology of MSCs and their potential use for therapy, there is still no generally accepted protocol for isolating and expanding MSCs in culture." This application disclosed that "in one preferred embodiment of the present invention, the isolated mesenchymal stem cells proliferate without differentiation and reach confluence even after 12 passages. The cell populations having greater than 98% homogeneous MSCs are obtained in accordance with the method of the present invention." [0031] The unexpected result is supported by post-filing art (Kato et al US Patent Application 20050013804, filing date: 09/12/2001), which mentioned that "The conventional culture methods however cannot produce sufficient amounts of mesenchymal stem cells because the proliferation of said stem cells stops or becomes extremely slow around 15th generation."

Remark to the Pre-Appeal Brief Review Summary

The Pre-Appeal Brief Review stated that

> Prockop et al teach a method that allows a greater number of MSCs to be generated, including a greater percentage of differentiatable (i.e., multipotential cells) than prior art culture methods, one would have expected the culture conditions taught by Prockop et al to provide culture to confluence for 12 passages without differentiation.

However, evidence (Blood, 2011 Vol. 117 No 2, pp 459-469; Stem cells, 2004 Vol. 22, Issue 6, pages 1123–1125) clearly demonstrates that the low density can not be extended for up to 6-7 passages. Cells undergo senescence. In other words, based on these evidence, one with ordinary skill in the art would not have expected the culture conditions taught by Prockop to provide culture to confluence for 12 passages without differentiation.

(viii) *Claims appendix.*

1. A method for isolating mesenchymal stem cells from bone marrow aspirate, comprising:

(a) providing a cell mixture comprising mesenchymal stem cells and other cells;

(b) seeding and culturing the cell mixture in a culture device comprising an upper plate with pores and a lower plate base, said upper plate made of a mesenchymal stem cell adhering material with pores of about 0.4 to 40 microns in diameter, where mesenchymal stem cells adhere and are cultured, and the lower plate base, where small-sized cells adhere following passing through the pores in

the upper plate, said culturing with medium containing factors that stimulate mesenchymal stem cells growth without differentiation and allow for the selective adherence of only the mesenchymal stem cells to the upper plate surface;

(c) purifying mesenchymal stem cells by removal of haematopoietic stem cells and non-adherent cells on the upper plate by changing medium; and

(d) collecting mesenchymal stem cells from the upper plate by recovering with trypsin-EDTA.

4. The method as claimed in claim 1, wherein the cell mixture comprises mammalian mesenchymal stem cells.

6. The method as claimed in claim 4, wherein the cell mixture comprises human mesenchymal stem cells.

9. The method as claimed in claim 1, wherein the mesenchymal stem cells can differentiate into tissues comprising bone, adipose, or cartilage.

10. The method as claimed in claim 1, wherein the mesenchymal stem cells are characterized by CD34-.

11. The method as claimed in claim 1, wherein the culture medium is 10% fetal bovine serum-supplemented Dulbecco's modified Eagle's medium containing 1 g/L of glucose.

34. The method as claimed in claim 1, wherein the mesenchymal stem cell adhering material is plastic.

35. The method as claimed in claim 1, wherein the mesenchymal stem cells cultured until confluence.

38. The method as claimed in claim 35~~36~~, further comprising re-plating the cells to expand the mesenchymal stem cells at a density of 4×10^3-$10^4/cm^2$.

43. The mesenchymal stem cells obtained by the method as claimed in claim 1, wherein cell populations having greater than 98% homogeneous mesenchymal stem cells.

44. The mesenchymal stem cells obtained by the method as claimed in claim 1, wherein the mesenchymal stem cells proliferate without differentiation and

reach confluence after several passages.

45. The mesenchymal stem cells obtained by the method as claimed in claim 43, wherein the mesenchymal stem cells proliferate without differentiation and reach confluence after 12 passages.

(ix) *Evidence appendix*.

No evidence has been submitted during this prosecution of this application.

A Notice of Allowance is respectfully requested.

Respectfully submitted:
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